Environmental Health Perspectives
Vol. 38, pp. 111-117, 1981

Testicular Cyclic Nucleotide and Adrenal Catecholamine Metabolism Following Chronic Exposure to Cadmium

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Cadmium (Cd) produces injurious effects on reproductive function and has been implicated in the pathogeneses of hypertension. The present article summarizes available data on alterations in the cyclic AMP system of testicular and prostatic tissue as well as in catecholamine metabolism in adrenal glands following exposure to Cd and subsequent withdrawal. Daily Cd (1 mg/kg IP) for 45 days decreased prostatic and testicular weights of mature male rats. In prostate, chronic treatment with Cd reduced cyclic AMP levels to 57% of normal values which appeared to be due to the decrease in adenylate cyclase activity since cyclic AMP metabolism by phosphodiesterase was not significantly altered. Cyclic AMP binding to prostatic protein kinase was increased following Cd administration as was the activity of the cyclic AMP-dependent form of protein kinase. In contrast to the prostate, testicular adenylate cyclase was stimulated by Cd treatment. However, the endogenous cyclic AMP levels remained unaffected since the increase in testicular adenylate cyclase was offset by a concomitant increase in the activity of phosphodiesterase. Although the activities of the cyclic AMP-dependent and the independent forms of testicular protein kinase were significantly depressed, the binding of cyclic AMP to protein kinase from testes of Cd-treated rats was not affected. Discontinuation of treatment for 28 days in rats that had previously been given the heavy metal for 45 days resulted in at least a partial reversal of several of the cadmium-induced changes in cyclic AMP metabolism of the rat prostate and testes. However, the weight of the prostate glands remained essentially in the same range as that seen in the "treated group."

Data suggest that cyclic AMP metabolism in both the primary and the secondary reproductive organs is altered following chronic Cd treatment and that some changes persist even 28 days following the termination of daily exposure to the heavy metal.

Cd treatment also increased adrenal weights and augmented the levels of adrenal norepinephrine and epinephrine as well as the activity of tyrosine hydroxylase. Discontinuation of the heavy metal treatment for 28 days, in rats previously injected with Cd for 45 days, restored the activity of tyrosine hydroxylase as well as the amount of norepinephrine and epinephrine. In contrast, adrenal weights were restored only partially following withdrawal of Cd treatment. Evidence indicates that the changes in adrenal catecholamine metabolism may be the result of stress induced by chronic exposure to this heavy metal. In addition, some of the untoward effects such as hyperglycemia and arterial hypertension seen during Cd toxicity might be related to increased synthesis of epinephrine in adrenal glands.

Target organ toxicity, particularly that of environmental toxicology, deals with incidental exposure of biological tissues to chemicals that are contaminants of the environment, food or water. Toxicologists, endocrinologists and pharmacologists alike are engaged in the study of the causes, conditions, effects, limits of safety of such exposure to chemicals, as well as in elucidating the subcellular mechanisms which underline their overt toxic effects (*1*). In the field of heavy metal toxicity, cadmium, lead, and methylmercury appear to have attracted the greatest attention over the past 10 years. Marked disturbances in glucose homeostasis, testicular necrosis and atrophy, cardiac hypertrophy, renal dysfunction, liver damage, impaired pancreatic function, and retardation of growth rates are among some of the prominent toxic

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manifestations of exposure to cadmium. There is a worrisome void in our knowledge of the long-range consequences of Cd toxicity and I really think, Harvey (*2*) said it best: "Cadmium is not simply of academic interest. While its role as an aetiological agent in disease is still speculative, there is no doubt that the ionised form of the element is an extremely powerful poison of many basic animal enzyme systems. In man there is a causal link with renal lesions, lung disease, bone lesions and hypertensive vascular disease. We simply do not know how dangerous it is or how wide is the safety margin. It is alarming to learn that the cadmium content of the polluted Severn estuary amounts to 4-5 μg per liter. Clearly, urgent studies are needed lest we, like Cadmus, King of Thebes, are not in danger of sowing some very lethal dragon's teeth for our children to harvest. The Japanese pollution disaster should not pass unheeded in our cadmium-enriched society." Indeed, just last year, Ellis et al. (*3*) measured *in vivo* Cd levels in 20 male human volunteers using the noninvasive partial body neutron activation technique and showed that Cd levels in the left kidney and in the liver of smokers were significantly elevated over those of nonsmokers. In this article, I shall confine my remarks to a brief discussion of the influence of chronic Cd treatment on the cyclic AMP system of testes and prostate as well as on catecholamine levels and their synthesizing enzyme, tyrosine hydroxylase (TH) in adrenal glands.

Effects of Cd on the Reproductive System

Injury to Testicular and Prostatic Function

Acute exposure to large doses of Cd induces gonadal necrosis whereas chronic treatment with low doses has been shown to produce testicular atrophy (*4-6*). It was not until mid 1950's that Parizek (*7*) thoroughly studied the destructive effects of Cd on testicular tissue. He observed that following parenteral administration of Cd (2.2 mg/kg), the testes became swollen, dark red, or purple. The weight then rapidly decreased and the testicles turned small, hard and yellowish. Thus, the testes become hemorrhagic, edematous, and in time, necrotic. The sterilizing effect of Cd is very rapid, and animals can become permanently sterile as early as 24 hr after injection (*4, 7*). The testes seem to be extremely sensitive to this metal as hemorrhagic necrosis of rodent testes can be induced by Cd concentrations as low as 0.15 μg/g. In contrast, the presence of even greater concentrations of Cd in kidneys and liver failed to inflict similar necrotic changes in these tissues. Although the selective destruction of rat testis and its associated vasculature by Cd is now well documented, the precise mode of action of Cd in the gonad remains unresolved.

Protection against Cd-Induced Testicular Damage

Parizek also demonstrated that large doses of Zn salts could prevent the action of Cd on the testes (*7*). Similar findings have since been reported by several other authors (*5, 9-12*). Zinc deficiency in animals affects the gonads and it has been found that Cd administration can displace significant amounts of testicular Zn. Since Zn is essential for the maintenance of germinal epithelium, it has been suspected that Cd might exert its initial injurious effects on Zn-dependent spermatogenic elements. It was thus initially hypothesized that damaged spermatogenic epithelium releases substances from the degenerating cells and causes, as a secondary effect, edema and circulatory changes in the interstitium (*7*). However, the fact that Cd evoked typical hemorrhagic necrosis even in testes devoid of any germinal epithelium (as in the cryptorchid) would seem to oppose this hypothesis (*11*). On the other hand, Zn might be essential for normal metabolic activity and/or tissue integrity at some other testicular site(s), where Cd could be displacing Zn. It is interesting that the receptors on vasculature of the testis that are vulnerable to Cd do not appear until the animals are between 2 to 3 weeks old. At the same time, the blood vessels undergo maturation in known endocrine, biochemical, and physiological characteristics (*13*). In the newborn rat testis, the alkaline phosphatase activity as well as the barrier to the passage of dyes is virtually absent. By 20 days of age, however, the adult type of barrier is evident and alkaline phosphatase levels reach the adult value (*14, 15*). Unfortunately, very few studies on Cd-induced vascular injury have examined the biochemical alterations prior to the onset of generalized necrosis. In one study, succinic dehydrogenase activity of the internal spermatic artery-pampiniform plexus complex decreased 4 hr after the administration of Cd (*16*). In contrast, alkaline phosphatase is suspected to be involved with active transport across the capillary wall (*15*). The possibility that alkaline phosphatase provides free energy for selective permeability in mature testicular capillaries is compatible with the normal increase in its activity during maturation as active

transport decreases and the abnormal increase in its activity after Cd injury (*13, 17*) which occurs at the same time as the loss of permeability barriers. Dimow and Knorre (*18*) reported that 6 hr after Cd administration, there was a decrease in the activities of three enzymes that are primarily associated with seminiferous tubules. Activities of succinic dehydrogenase, lactic dehydrogenase, and diphosphopyridine nucleotide (DPN) diaphorase were depressed. It is of interest that the enzymes (alkaline phosphatase and dehydrogenases) which are altered by Cd, are Zn metalloenzymes, i.e., Zn-requiring enzymes. Hodgen et al. (*19*) studied the effects of Cd on another Zn-containing enzyme, carbonic anhydrase, in the testes and found a decrease in its activity as early as one-half hour after Cd administration. However, after 2 hr, carbonic anhydrase activity was increased and remained high throughout the experimental period. Hodgen et al. (*20*) described an isoenzyme of carbonic anhydrase which was thought to be specific to the testis as it was absent in both erythrocytes or kidneys. They suggested that this organ-specific carbonic anhydrase was the primary site of action of Cd on testicles. Activity of this isoenzyme was decreased as early as 30 min. after Cd injection. Conversely, testes taken from animals exposed to Cd for 4 hr contained no isoenzyme T, but exhibited demonstrable amounts of carbonic anhydrases I, II, and IV, apparently due to hemorrhage of the testis and consequent invasion of carbonic anhydrase-rich erythocyte into interstitial spaces (*19, 20*).

Zn is not the only element implicated in the etiology of Cd-induced testicular damage. Selenium has been found to be even more efficient than Zn in preventing Cd-induced gonadal damage (*9, 21, 22*). Initially, it was suspected that Se might be diverting Cd away from the testis, but it was later found that Se actually increased the testicular uptake of Cd. The mechanism by which Se affords protection against the necrotizing action of Cd is, of course, still obscure. Cadmium has high affinity for SH (sulfhydryl) groups, and SH inhibitors including cadmium chloride have been reported to induce selective necrosis in the β-cell region (*2, 3*). It has been postulated by Gunn et al. (*22*) that Se temporarily complexes with Cd preventing sufficient free Cd from reacting with testicular SH groups. Furthermore, it was observed that SH inhibitors reduced the amount of pancreatic Zn (*23*). Since Zn deficiency has been reported to result in gonadal damage, Se by protecting the SH groups from free Cd might be sparing Zn which is essential for normal testicular function. Other agents which protect against Cd-induced testicular injury include manganese, cobalt, copper, calcium, iron, lithium, nickel, arsenic, and uranium (*13*). Metallothionein has also been implicated to play a protective role against Cd-induced testicular necrosis. Nordberg et al. (*24*) were able to demonstrate that whereas Cd-metallothionein complex was without effect, a comparable dose of cadmium chloride administered by the same route resulted in testicular necrosis. It seems possible that certain elements might afford protection against Cd-toxicity by inducing metallothionein formation.

Effects of Cd Exposure on Fertility and Spermatogenesis

Lee and Dixon (*6*) reported that a single injection of Cd, at a dose that did not cause any morphological change in testicular vasculature, produced a significant decrease in fertility for a period of 55 days. They were also able to demonstrate that late elongated spermatids had an affinity for Cd that was 2.5-2.9 times that of Zn at equimolar concentrations; the incorporation of these two metals into the late elongated spermatids appeared to be competitive. Furthermore, Cd administration (1.0 mg/kg, IP) inhibited thymidine uptake into spermatogonial cells by 42 and 52% of the control values on days 2 and 7, respectively. Pretreatment of mice with $ZnCl_2$ (1 mg/kg) prior to Cd treatment completely blocked the biochemical and functional effects of Cd on spermiogenic cells but not on spermatogonial cells. Since the effect of Cd on spermatogonia, where DNA synthesis occurs, was not reversed by Zn, Lee and Dixon (*6*) postulated that this persistent Cd-induced biochemical lesion might be due to the inhibitory effect of Cd on DNA (deoxyribonucleic acid) synthesis. This is supported by the fact that Cd interacts with both phosphate and base sites on DNA, thus affecting the unwinding and rewinding of DNA, the processes essential for replication and transcription. However, this is only a postulation and more work is required in this area. Nevertheless, the most likely action of Cd is a direct interaction of Cd with DNA and competitive inhibition of essential enzymes. The first action is not reversed by Zn while the second one is.

So far, we have considered only the effects of a single dose of Cd. However, from the point of view of environmental toxicologists, acute effects of large doses may be only of limited interest. Histological changes in the testicles of rats as a result of long-term administration of Cd in the food were reported by Pindborg (*25*) and Ribelin (*26*). Pindborg(*25*) gave doses of 0.025-0.1% $CdCl_2$ (150-610 ppm of Cd) and Ribelin (*26*), 50-1270 ppm of Cd in the diet. The changes reported by Ribelin (*26*) were

different from those developing after a single injection, but were not different from those produced by several other toxic substances tested. Richardson et al. (*27*) studied the histological sequelae of feeding 75 mg Cd/kg of diet from hatching to 4 or 6 weeks in Japanese quail. Amongst other effects, testicular hypoplasia was one of the most obvious lesions produced by dietary Cd. It is of interest that similar failure of normal spermatogenic development was also observed in birds weaned on Zn-deficient diet, suggesting that Cd may interfere with Zn metabolism (*27*). On the other hand, Piscator and Axelsson (*28*) could not observe any histological abnormalities in testicles of rabbits injected with Cd for 24 weeks and maintained for another 30 weeks without any Cd administration. They suggested that absence of testicular changes could be due to the relative insensitivity of rabbits to the necrotizing action of Cd, or to formation of metallothionein in the liver. It is of interest that repetitive injection of small quantities of Cd into mice increased the amount of Cd in the testes without inducing degenerative changes, and furthermore, subsequent exposure of these animals to normally toxic doses of Cd produced no testicular damage (*29*).

Cd and the Cyclic AMP System

Cyclic nucleotides are now known to be involved in almost every aspect of the reproductive process. Recently, Sanborn, Heindel, and Robison (*30*) reviewed the role of cyclic AMP in the synthesis and release of gonadotrophins, the regulation of gonadal function, the function of spermatozoa and the maintenance of reproductive tract function. In order to further elucidate the effects of chronic Cd on the male reproductive organs, we examined its influence on the cyclic AMP system of rat prostate and testicular tissues (*31*).

Our experiments demonstrated that daily IP injections of cadmium chloride (1 mg/kg) for 45 days decreased prostatic and testicular weights of mature male rats (Table 1). In the prostate gland, chronic treatment with Cd reduced cyclic AMP levels to 57% of normal values, which appeared to be due to the decrease in adenylate cyclase activity since cyclic AMP metabolism by phosphodiesterase was not significantly altered. Cyclic AMP binding to prostatic protein kinase was increased following Cd administration, as was the activity of the cyclic AMP-dependent form of protein kinase. In contrast to the prostate, testicular adenylate cyclase was stimulated by Cd. However, the endogenous cyclic AMP levels remained unaffected since the increase in testicular adenylate cyclase was offset by a concomitant increase in the activity of phosphodiesterase. Although the activities of the cyclic AMP-dependent and -independent forms of testicular protein kinase were significantly depressed, the binding of cyclic AMP to protein kinase from testes of Cd-treated rats was not affected.

Discontinuation of treatment for 28 days in rats that had previously been given cadmium for 45 days resulted in a reversal of several of the Cd-induced changes in cyclic AMP metabolism of the prostate gland. However, the weight of the prostate glands remained essentially in the same range as that seen in the group "treated" for 45 days. In the case of testes, cessation of Cd treatment restored adenylate cyclase and protein kinase (the cyclic AMP-dependent form) activities back to normal. However, endogenous cyclic AMP levels, the cyclic AMP binding capacity of protein kinase, as well as testicular phosphodiesterase and the cyclic AMP-independent form of protein kinase were still significantly reduced in the "withdrawal group" (Fig. 1). Data suggest that cyclic AMP metabolism in both the primary and the secondary reproductive organs of the male rat is altered following chronic Cd treatment and that the metabolic changes persist even 28 days following the termination of daily exposure to the heavy metal (*31*).

The available reports indicate that acute or chronic exposure of experimental animals to Cd can induce biochemical and/or functional alterations in male reproductive organs. Furthermore, observations of a great number of animal species indicate that similar effects are likely to occur in humans (*32*). So far, however, testicular necrosis as a result

Table 1. Effects of Cd treatment on body, testicular and prostatic weights.[a]

Parameters	Control	Treated	Withdrawn
Body weight, g	363 ± 10	302 ± 26[b]	365 ± 11
Testes, g	3.2 ± 0.1	2.1 ± 0.[b]	2.0 ± 0.1[b]
Prostate, mg	465 ± 24	279 ± 27[b]	297 ± 35[b]

[a]Each value represents the mean ± SEM of six rats in each group. Twelve animals were given 1 mg/kg dose of cadmium chloride daily by the intraperitoneal route for 45 days. One half of the animals were killed 24 hr after the last injection (treated group). The other 6 rats were maintained for an additional period of 28 days without any treatment and constituted the withdrawal group.
[b]Statistically significant difference in comparison with the values of control rats ($p < 0.05$).

of Cd exposure has not been reported for humans. Industrially exposed workers do seem to accumulate considerable amounts of Cd in the testis and some histological changes of rather unspecific nature have been noted upon postmortem examination (*33*). In addition, Favino et al. (*34*) investigated fertility of 10 Cd-exposed workers and found only one case of impotency with abnormally low urinary testosterone levels. It is therefore obvious that further studies need to be undertaken on the chronic effects of Cd on reproductive function in both experimental animals and industrially exposed humans.

Alterations in Adrenal Catecholamine Metabolism Following Cd Exposure

Although biomedical investigators have made great advances in treating infectious diseases, the picture is not as bright with respect to cardiovascular disorders. The term "cardiovascular diseases" includes such conditions as coronary and cerebral thromboses and infarcts, cerebral ischemia, angina pectoris, and of course hypertension. In the context of this review, it is to be recognized that hypertension may be the penultimate factor involved in the progression of other diseases of the heart, blood vessels and kidneys. It is well established that hypertension accelerates the atherosclerotic process and the root cause of congestive heart failure is hypertension of long-standing duration. It is no surprise that one of the highest medical priorities today is delineation of specific factors underlying the pathogenesis of hypertension (*35*).

Reports continue to appear that Cd produces hypertension. Carroll (*36*) observed a positive correlation between the Cd content of air and the incidence of hypertension and arteriosclerosis in 28 North American cities. Although the importance of catecholamines in the etiology of hypertension has long been appreciated, the exact relationship is still not fully understood (*37*). The observation that Cd produces hyperglycemia (*38*) and that it might be an etiological factor in the development of hypertension (*39*, *40*) prompted us to investigate the influence of this metal on catecholamine metabolism of rat adrenal glands.

Daily injection of cadmium chloride (1 mg/kg IP) for 45 days significantly increased adrenal weights and augmented the levels of adrenal norepinephrine and epinephrine as well as the activity of adrenal tyrosine hydroxylase (TH). Discontinuation of the treatment for 28 days, in rats previously injected with Cd for 45 days, restored the activity

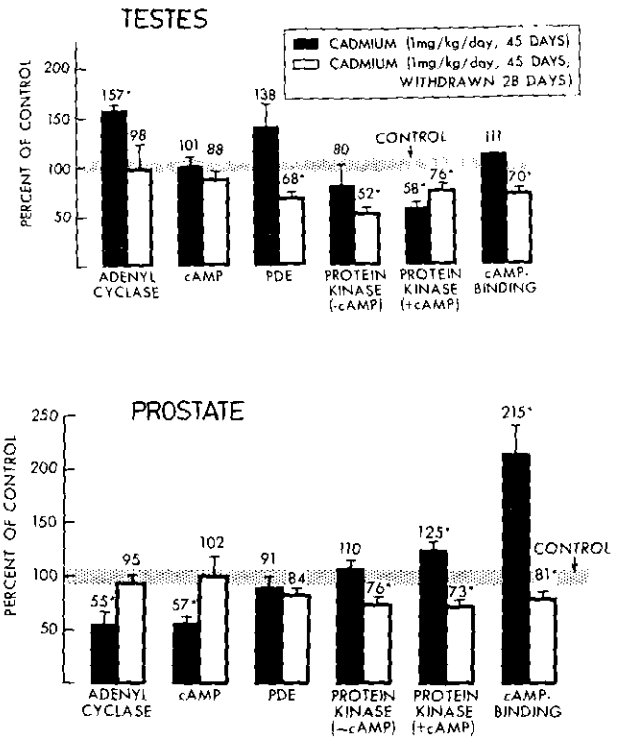

FIGURE 1. The influence of cadmium on cyclic AMP metabolism and protein kinase activity of rat testes and prostate. Each bar represents the mean ± S.E.M. of 6 rats in each group. Data are given in percentages taking the values of control animals as 100%. Twelve animals were given cadmium chloride daily by the intraperitoneal route in a dose of 1 mg/kg for 45 days. One half of the animals were killed 24 hr after the last injection (treated group). The remaining 6 rats were maintained for an additional period of 28 days without any treatment (withdrawn group). The asterisks (*) denote a statistically significant difference when compared with the values of control rats ($p < 0.05$).

of TH as well as the amount of norepinephrine and epinephrine (Table 2). In contrast, adrenal weights were restored only partially following the withdrawal of Cd treatment (*41*).

Our data demonstrate that chronic exposure to cadmium significantly enhances adrenal weights and produces changes in the metabolism of adrenal catecholamine. The cessation of heavy metal treatment for 28 days in rats previously exposed to cadmium, restored the activity of adrenal TH as well as the levels of norepinephrine and epinephrine to almost normal. The increase in adrenal weights after prolonged treatment with cadmium is in accord with early observations corroborating the idea that these endocrine glands play an important role in response to exposure to potentially toxic agents. The dose- and time-dependent increases in the levels of adrenal norepinephrine and epineph-

Table 2. Effects of 45 days Cd treatment and subsequent withdrawal on tyrosine hydroxylase, norepinephrine, and epinephrine levels in adrenal glands.[a]

Treatment	Adrenal wt, mg	TH, nmole DOPA/g wet tissue/hr	NE, μg/g	E, μg/g
Control	37 ± 3	567 ± 75	66.6 ± 6	373.1 ± 37
Treated	53 ± 4[b]	887 ± 83[b]	102.2 ± 2[b]	496.2 ± 29[b]
Control	39 ± 4	585 ± 57	73.4 ± 12	271.9 ± 41
Withdrawn	49 ± 4[b]	562 ± 49[c]	73.4 ± 10	409.1 ± 44

[a] Values represent the means ± SEM of six animals in each group. Cadmium chloride (1 mg/kg) was injected daily for 45 days by the intraperitoneal route. A group of rats which had been given Cd for 45 days was maintained without any treatment for an additional period of 28 days. Abbreviations are: TH, tyrosine hydroxylase; NE, norepinephrine; E, epinephrine.

[b] Statistically significant difference when compared with the values of control rats ($p < 0.05$).

[c] Statistically significant difference when compared with the values of cadmium-treated rats ($p < 0.05$).

rine in cadmium augmented the activity of TH, the enzyme which plays a rate-limiting role in the biosynthesis of CA. The present results seem to be consistent with previous data demonstrating an increase in the activity of this adrenal enzyme following drug-induced sympathoadrenal hyperactivity (*42*, *43*). In addition, Klain found significant increases in the activities of TH, dopamine β-hydroxylase and phenyl-*N*-methyltransferase in rats subjected to alarming stimulus such as high altitude stress (*37*).

The elevated epinephrine content together with increased norepinephrine levels may be related to the effect of cadmium on adrenal corticoids which have been shown to induce the activity of phenylethanolamine *N*-methyl-transferase (*44*, *45*), the enzyme responsible for methylating norepinephrine to epinephrine. However, further work is necessary to answer the question whether cadmium produces changes in adrenal cortical activity which might be relevant to the effect of this heavy metal on adrenal catecholamine metabolism. Since both immobilization and cold stress augment sympathetic nervous system activity as well as adrenal TH (*46*, *47*), the changes observed in this study could be related to the stress induced by chronic exposure to cadmium. The hyperglycemia seen during cadmium treatment (*38*) may be the reflection of increased synthesis of adrenal epinephrine, which might also be associated with the known cardiovascular effects of cadmium toxicity.

In conclusion, from the point of view of target organ toxicity with reference to the endocrine system, both adrenal glands and reproductive tissues present exciting possibilities for investigating the subcellular sites of functional alterations that are associated with exposure to environmental pollutants.

The author acknowledges with gratitude the valuable contributions of Drs. Z. Merali and R. B. Rastogi. This research was supported by grants from the Medical Research Council and National Science and Engineering Research Council of Canada.

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